

Applicant's or agent's  
file reference

31085

International application No.

PCT/FI99/00185

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description  
on page 55, line S 14, 15 and 16

**B. IDENTIFICATION OF DEPOSIT**

Further deposits are identified on an additional sheet

Name of depositary institution

DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

Address of depositary institution (*including postal code and country*)

Mascheroder Weg 1b, D-38124 Braunschweig, Germany

Date of deposit

5 March 1999

Accession Number

DSM 12722, DSM 12723, DSM 12724

**C. ADDITIONAL INDICATIONS (leave blank if not applicable)**

This information is continued on an additional sheet

In respect of those designations in which a European patent or a patent in Canada, Iceland or Norway is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or the corresponding information concerning the patent in Canada, Iceland or Norway or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the Commissioner (in Canada) or the person requesting the sample (Rule 28(4) EPC and the corresponding regulations in Canada, Iceland and Norway).

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)**

**E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)**

The indications listed below will be submitted to the International Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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28 APRIL 1999

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*A. Nickel*

Applicant's or agent's file reference	31085	International application No.	PCT/FI99/00185
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<p>A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>55</u>, line S <u>14, 15 and 16</u></p>			
<b>B. IDENTIFICATION OF DEPOSIT</b>		<small>Further deposits are identified on an additional sheet</small> <input checked="" type="checkbox"/>	
<p>Name of depositary institution <b>DSMZ–Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH</b></p>			
<p>Address of depositary institution (<i>including postal code and country</i>)  Mascheroder Weg 1b, D-38124 Braunschweig, Germany</p>			
<p>Date of deposit <b>5 March 1999</b></p>	<p>Accession Number <b>DSM 12722, DSM 12723, DSM 12724</b></p>		
<b>C. ADDITIONAL INDICATIONS</b> ( <i>leave blank if not applicable</i> )		<small>This information is continued on an additional sheet</small> <input type="checkbox"/>	
<p>When designating Australia, in accordance with regulation 3.25 of the Patents Regulations (Australia Statutory Rules 1991 No. 71), samples of materials deposited in accordance with the Budapest Treaty in relation to this Patent Request are only to be provided before: the patent is granted on the application; or the application has lapsed or been withdrawn or refused; to a person who is: a skilled addressee without an interest in the invention; and nominated by a person who makes a request for the furnishing of those samples.</p>			
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> ( <i>if the indications are not for all designated States</i> )			
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> ( <i>leave blank if not applicable</i> )			
<p>The indications listed below will be submitted to the International Bureau later (<i>specify the general nature of the indications e.g., "Accession Number of Deposit"</i>)</p>			
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<p>Authorized officer</p>		<p>Authorized officer</p> <p><i>A. Neckl</i></p>	

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INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 55, lines 11, 12 and 13

B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet

Name of depositary institution

DSMZ—Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

Address of depositary institution (*including postal code and country*)

Mascheroder Weg 1b, D-38124 Braunschweig, Germany

Date of deposit

4 June 1998

Accession Number

DSM 12213, DSM 12214, DSM 12215

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*)

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In respect of those designations in which a European patent or a patent in Canada, Iceland or Norway is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or the corresponding information concerning the patent in Canada, Iceland or Norway or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the Commissioner (in Canada) or the person requesting the sample (Rule 28(4) EPC and the corresponding regulations in Canada, Iceland and Norway).

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E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the International Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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Applicant's or agent's file reference	31085	International application No. PCT/FI99/00185
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**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
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A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>55</u> , lines <u>11, 12 and 13</u>	
B. IDENTIFICATION OF DEPOSIT	
Name of depositary institution <b>DSMZ–Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH</b>	
Address of depositary institution ( <i>including postal code and country</i> )  Mascheroder Weg 1b, D-38124 Braunschweig, Germany	
Date of deposit  4 June 1998	Accession Number  <b>DSM 12213, DSM 12214, DSM 12215</b>
C. ADDITIONAL INDICATIONS ( <i>leave blank if not applicable</i> )	
When designating Australia, in accordance with regulation 3.25 of the Patents Regulations (Australia Statutory Rules 1991 No. 71), samples of materials deposited in accordance with the Budapest Treaty in relation to this Patent Request are only to be provided before: the patent is granted on the application; or the application has lapsed or been withdrawn or refused; to a person who is: a skilled addressee without an interest in the invention; and nominated by a person who makes a request for the furnishing of those samples.	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE ( <i>if the indications are not for all designated States</i> )	
E. SEPARATE FURNISHING OF INDICATIONS ( <i>leave blank if not applicable</i> )	
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A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>55</u> line S <u>24</u> and <u>25</u>	
B. IDENTIFICATION OF DEPOSIT	
Name of depositary institution <b>DSMZ–Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH</b>	
Address of depositary institution ( <i>including postal code and country</i> )  <b>Mascheroder Weg 1b, D-38124 Braunschweig, Germany</b>	
Date of deposit <b>11 March 1999</b>	Accession Number <b>DSM 12728, DSM 12729</b>
C. ADDITIONAL INDICATIONS ( <i>leave blank if not applicable</i> )	
In respect of those designations in which a European patent or a patent in Canada, Iceland or Norway is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or the corresponding information concerning the patent in Canada, Iceland or Norway or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the Commissioner (in Canada) or the person requesting the sample (Rule 28(4) EPC and the corresponding regulations in Canada, Iceland and Norway).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE ( <i>if the indications are not for all designated States</i> )	
E. SEPARATE FURNISHING OF INDICATIONS ( <i>leave blank if not applicable</i> )	
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**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>55</u> line S <u>24 and 25</u>	
B. IDENTIFICATION OF DEPOSIT	
Name of depositary institution <b>DSMZ–Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH</b>	
Address of depositary institution ( <i>including postal code and country</i> )  <b>Mascheroder Weg 1b, D-38124 Braunschweig, Germany</b>	
Date of deposit  <b>11 March 1999</b>	Accession Number  <b>DSM 12728, DSM 12729</b>
C. ADDITIONAL INDICATIONS ( <i>leave blank if not applicable</i> )	
This information is continued on an additional sheet <input type="checkbox"/>  When designating Australia, in accordance with regulation 3.25 of the Patents Regulations (Australia Statutory Rules 1991 No. 71), samples of materials deposited in accordance with the Budapest Treaty in relation to this Patent Request are only to be provided before: the patent is granted on the application; or the application has lapsed or been withdrawn or refused; to a person who is: a skilled addressee without an interest in the invention; and nominated by a person who makes a request for the furnishing of those samples.	
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Authorized officer  <i>A. Neel</i>	



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :  C12N 1/19, 1/21, C12P 7/10, 7/42, 13/08		A1	(11) International Publication Number: <b>WO 99/46363</b>  (43) International Publication Date: 16 September 1999 (16.09.99)
<p>(21) International Application Number: <b>PCT/FI99/00185</b></p> <p>(22) International Filing Date: 11 March 1999 (11.03.99)</p> <p>(30) Priority Data: 980551 11 March 1998 (11.03.98) FI</p> <p>(71) Applicant (for all designated States except US): VALTION TEKNILLINEN TUTKIMUSKESKUS [FI/FI]; Vuorimiehentie 5, FIN-02150 Espoo (FI).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): ARISTIDOU, Aristos [CY/FI]; Takojantie 1 N, FIN-02130 Espoo (FI). LONDERSBOROUGH, John [FI/FI]; Jaakkärinkatu 9 A 15, FIN-00150 Helsinki (FI). PENTTILA, Merja [FI/FI]; Vattuniemenskatu 2 A 22, FIN-00210 Helsinki (FI). RICHARD, Peter [DE/FI]; Mannerheimintie 51 A 10, FIN-00250 Helsinki (FI). RUOHONEN, Laura [FI/FI]; Runeberginkatu 35 A 9, FIN-00100 Helsinki (FI). SÖDERLUND, Hans [FI/FI]; Salontie 19, FIN-02940 Espoo (FI). TELEMAN, Anita [SE/SE]; Belestigen 11, S-182 64 Djursholmen (SE). TOIVARI, Mervi [FI/FI]; Kukkumäenkuja 12 as 1, FIN-02280 Espoo (FI).</p> <p>(74) Agent: OY JALO ANT-WUORINEN AB; Iso Roobertinkatu 4-6 A, FIN-00120 Helsinki (FI).</p>		<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPo patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments. With an indication in relation to deposited biological material furnished under Rule 13bis separately from the description.</p>	
<p>(54) Title: TRANSFORMED MICROORGANISMS WITH IMPROVED PROPERTIES</p> <p>(57) Abstract</p> <p>The present invention relates to genetic engineering of production microorganisms used in biotechnology to improve their properties so that they produce useful products more efficiently. The microorganisms express at least one enzyme that causes the functional coupling of the oxidation and reduction of substrates by two pyridine nucleotide-linked dehydrogenase reactions with different specificities for the NAD/NADH and NADP/NADPH coenzyme couples and so facilitates the transfer of electrons between the two coenzyme couples through the said substrates. In particular the invention relates to increasing the yields of products such as ethanol or amino acids from carbon and nitrogen sources such as biomass comprising hexoses, pentoses or their polymers.</p>			

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 99/00185

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 1/19, C12N 1/21, C12P 7/10, C12P 7/42, C12P 13/08  
 According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N, C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, MEDLINE, WPI

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Microbiology, Volume 142, 1996, Nina Meinander et al, "A heterologous reductase affects the redox balance of recombinant <i>Saccharomyces cerevisiae</i> ", page 165 - page 172, see esp. fig 3 and table 2  --	1-38
A	EP 0733712 A1 (AJINOMOTO CO., INC.), 25 Sept 1996 (25.09.96)  --	1-38
A	WO 9742307 A1 (PURDUE RESEARCH FOUNDATION), 13 November 1997 (13.11.97)  -----	1-38

Further documents are listed in the continuation of Box C.

See patent family annex.

- \* Special categories of cited documents
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 June 1999	12-07-1999
Name and mailing address of the ISA / Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Authorized officer  Hampus Rystedt/Els Telephone No. + 46 8 782 25 00

## INTERNATIONAL SEARCH REPORT

Information on patent family members

03/05/99

International application No.

PCT/FI 99/00185

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0733712 A1	25/09/96	AU 687458 B AU 8002694 A BR 9407907 A JP 4324737 A PL 314090 A SK 53796 A US 5830716 A CA 2175042 A CN 1139956 A CZ 9601213 A HU 74840 A HU 9601085 D JP 2817400 B WO 9511985 A	26/02/98 22/05/95 26/11/96 13/11/92 19/08/96 01/10/96 03/11/98 04/05/95 08/01/97 11/09/96 28/02/97 00/00/00 30/10/98 04/05/95
WO 9742307 A1	13/11/97	AU 2830197 A EP 0898616 A	26/11/97 03/03/99

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/07663

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, Dialog files 301, 155, 5, 351 (Chemname, Medline, Biosis, Derwent WPI)  
search terms: Xyulokinase, xylrose reductase, xylitol dehydrogenase, vector?, plasmid?, (ribosom? or 18S or  
28S(3n)(gene? ? or chromosom? or locus or loci or cistron), integral?, insert?, yeast, *Saccharomyces*, *Candida*, *Pichia*,  
*Pachysolen*, ferment? degrad?, xylose, nonselect?

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/07663

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FUJII et al. Application of a Ribosomal DNA Integration Vector in the Construction of a Brewer's Yeast Having $\alpha$ -Acetolactate Decarboxylase Activity. Applied and Environmental Microbiology. April, 1990. Vol. 56. No. 4, pages 997-1003, entire document.	14-16, 18- 19, 28, 30 -----
Y	TANTIRUNGKIJ et al. Fed-batch fermentation of xylose by a fast-growing mutant of xylose-assimilating recombinant <i>Saccharomyces cerevisiae</i> . Applied Microbiology and Biotechnology. 1994. Vol. 41. No.1. pages 8-12, entire document.	1-13, 17, 20-27, 29
Y	TANTIRUNGKIJ et al. Genetic Improvement of <i>Saccharomyces cerevisiae</i> for Ethanol Production from Xylose. Ann. N. Y. Acad. Sci. 02 May 1994, Vol. 721, pages 138-147, entire document.	1-29
Y	WO 95/13362 A1 (PURDUE RESEARCH FOUNDATION) 18 May 1995. entire document.	1-29
X	LOPES et al. Factors affecting the mitotic stability of high copy number integration into the ribosomal DNA of <i>Saccharomyces cerevisiae</i> . Yeast. 1996. Vol. 12. pages 467-477, entire document.	14-16, 18-19, 28, 30 -----
Y		1-13, 17, 20-27, 29

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>31085</b>	<b>FOR FURTHER ACTION</b>	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. <b>PCT/FI 99/00185</b>	International filing date ( <i>day/month/year</i> ) <b>11 March 1999</b>	(Earliest) Priority Date ( <i>day/month/year</i> ) <b>11 March 1998</b>
Applicant <b>Valtion teknillinen tutkimuskeskus et al</b>		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1.  Certain claims were found unsearchable (See Box I).
2.  Unity of invention is lacking (See Box II).
3.  The international application contains disclosure of a nucleotide and/or amino acid sequence listing and the international search was carried out on the basis of the sequence listing
  - filed with the international application.
  - furnished by the applicant separately from the international application,
    - but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
  - transcribed by this Authority.
4. With regard to the title,  the text is approved as submitted by the applicant.
  the text has been established by this Authority to read as follows:
5. With regard to the abstract,
  - the text is approved as submitted by the applicant.
  - the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. The figure of the drawings to be published with the abstract is:
 

Figure No. \_\_\_\_\_

  - as suggested by the applicant.
  - because the applicant failed to suggest a figure.
  - because this figure better characterizes the invention.

None of the figures.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 99/00185

## Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

The present invention relates to genetic engineering of production microorganisms used in biotechnology to improve their properties so that they produce useful products more efficiently. The microorganisms express at least one enzyme that causes the functional coupling of the oxidation and reduction of substrates by two pyridine nucleotide-linked dehydrogenase reactions with different specificities for the NAD/NADH and NADP/NADPH coenzyme couples and so facilitates the transfer of electrons between the two coenzyme couples through the said substrates. In particular the invention relates to increasing the yields of products such as ethanol or amino acids from carbon and nitrogen sources such as biomass comprising hexoses, pentoses on their polymers.